

# UC San Diego

## UC San Diego Previously Published Works

### Title

Clinical Model for NASH and Advanced Fibrosis in Adult Patients With Diabetes and NAFLD: Guidelines for Referral in NAFLD.

### Permalink

<https://escholarship.org/uc/item/2hm9g11p>

### Journal

Diabetes care, 38(7)

### ISSN

0149-5992

### Authors

Bazick, Jessica  
Donithan, Michele  
Neuschwander-Tetri, Brent A  
et al.

### Publication Date

2015-07-01

### DOI

10.2337/dc14-1239

Peer reviewed



# Clinical Model for NASH and Advanced Fibrosis in Adult Patients With Diabetes and NAFLD: Guidelines for Referral in NAFLD

Diabetes Care 2015;38:1347–1355 | DOI: 10.2337/dc14-1239

Jessica Bazick,<sup>1</sup> Michele Donithan,<sup>2</sup>  
Brent A. Neuschwander-Tetri,<sup>3</sup>  
David Kleiner,<sup>4</sup> Elizabeth M. Brunt,<sup>5</sup>  
Laura Wilson,<sup>2</sup> Ed Doo,<sup>6</sup> Joel Lavine,<sup>7</sup>  
James Tonascia,<sup>2</sup> and Rohit Loomba,<sup>1,8\*</sup>

## OBJECTIVE

Approximately 18 million people in the U.S. have coexisting type 2 diabetes and nonalcoholic fatty liver disease (NAFLD). It is not known who among these patients has nonalcoholic steatohepatitis (NASH) with advanced fibrosis. Therefore, we aimed to determine factors that are associated with both NASH and advanced fibrosis in patients with diabetes and NAFLD in order to identify who should be prioritized for referral to a hepatologist for further diagnostic evaluation and treatment.

## RESEARCH DESIGN AND METHODS

This study was derived from the NASH Clinical Research Network studies and included 1,249 patients with biopsy-proven NAFLD (including a model development cohort of 346 patients and an independent validation cohort of 100 patients with type 2 diabetes as defined by the American Diabetes Association criteria). Outcome measures were presence of NASH or advanced fibrosis (stage 3 or 4) using cross-validated, by jackknife method, multivariable-adjusted area under the receiver operating characteristic curve (AUROC) and 95% CI.

## RESULTS

The mean  $\pm$  SD age and BMI of patients with diabetes and NAFLD was  $52.5 \pm 10.3$  years and  $35.8 \pm 6.8$  kg/m<sup>2</sup>, respectively. The prevalence of NASH and advanced fibrosis was 69.2% and 41.0%, respectively. The model for NASH included white race, BMI, waist, alanine aminotransferase (ALT), Aspartate aminotransferase (AST), albumin, HbA<sub>1c</sub>, HOMA of insulin resistance, and ferritin with an AUROC of 0.80 (95% CI 0.75–0.84,  $P = 0.007$ ). The specificity, sensitivity, negative predictive values (NPVs), and positive predictive values (PPVs) were 90.0%, 56.8%, 47.7%, and 93.2%, respectively, and the model correctly classified 67% of patients as having NASH. The model for predicting advanced fibrosis included age, Hispanic ethnicity, BMI, waist-to-hip ratio, hypertension, ALT-to-AST ratio, alkaline phosphatase, isolated abnormal alkaline phosphatase, bilirubin (total and direct), globulin, albumin, serum insulin, hematocrit, international normalized ratio, and platelet count with an AUROC of 0.80 (95% CI 0.76–0.85,  $P < 0.001$ ). The specificity, sensitivity, NPV, and PPV were 90.0%, 57%, 75.1%, and 80.2%, respectively, and the model correctly classified 76.6% of patients as having advanced fibrosis. Results remained consistent for both models in the validation cohort. The proposed model performed better than the NAFLD fibrosis score in detecting advanced fibrosis.

## CONCLUSIONS

Routinely available clinical variables can be used to quantify the likelihood of NASH or advanced fibrosis in adult diabetic patients with NAFLD. The clinical models presented can be used to guide clinical decision making about referrals of patients with diabetes and NAFLD to hepatologists.

<sup>1</sup>Department of Medicine, University of California, San Diego, La Jolla, CA

<sup>2</sup>Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD

<sup>3</sup>Division of Gastroenterology and Hepatology, Saint Louis University, St. Louis, MO

<sup>4</sup>Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD

<sup>5</sup>Department of Pathology and Immunology, Washington University in St. Louis School of Medicine, St. Louis, MO

<sup>6</sup>Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD

<sup>7</sup>Division of Pediatric Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, Columbia University, New York, NY

<sup>8</sup>Divisions of Gastroenterology and Epidemiology, University of California, San Diego, La Jolla, CA

Corresponding author: Rohit Loomba, roloomba@ucsd.edu.

Received 15 May 2014 and accepted 6 March 2015.

This article contains Supplementary Data online at <http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc14-1239/-/DC1>.

A slide set summarizing this article is available online.

\*R.L. participated on behalf of NASH CRN, sponsored by the NIDDK and NIH. The NASH CRN roster can be found in the Supplementary Data online.

© 2015 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered.

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the U.S. (1–3). Approximately 10–22% of patients with NAFLD have the progressive subtype of NAFLD termed nonalcoholic steatohepatitis (NASH), which can result in cirrhosis, hepatocellular carcinoma, and liver-related mortality (4–9). Type 2 diabetes is considered a major risk factor for advanced liver disease in patients with NAFLD (10–12).

It is estimated that ~25.8 million Americans are afflicted with diabetes (13–15). Several studies have shown that prevalence of NAFLD in patients with diabetes is increased compared with those without diabetes (16–21). Although the exact prevalence of NAFLD in patients with diabetes is not known, previous studies suggest that it ranges between 49.6 and 74% (17,21–25). Therefore, it can be estimated that ~13–18 million people in the U.S. have coexisting diabetes and NAFLD. This study aimed to address the clinical risk stratification that may be applied to this group of patients.

The presence of diabetes has consistently been shown to be a key predictor of NASH and advanced fibrosis in NAFLD (11,26,27). Several experts have recommended liver biopsy in select NAFLD patients with diabetes (28–32). In this era of accountable health care and increasing cost constraints, it is not feasible to recommend liver biopsy in all patients who have diabetes and NAFLD. For that reason, we aimed to determine the most reliable factors that are associated with NASH or advanced fibrosis in patients with diabetes and NAFLD in order to identify patients who should be prioritized for a liver biopsy and/or referred to a hepatologist for further evaluation.

## RESEARCH DESIGN AND METHODS

### Study Design, Setting, and Participants

This is a cross-sectional analysis of prospectively evaluated adult patients with biopsy-proven NAFLD who were enrolled into the NAFLD Database Study, a prospective cohort study, conducted by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-sponsored Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) (33,34). The NASH CRN studies enrolled 1,266 patients with biopsy-proven NAFLD; patients

enrolled through June 2012 were included in this analysis (33,34). Participants were enrolled by one of the eight participating medical centers in the U.S.: University of California, San Diego (La Jolla, CA); Duke University (Durham, NC); Case Western Reserve University/Cleveland Clinic Foundation (Cleveland, OH); Indiana University (Indianapolis, IN); Saint Louis University (St. Louis, MO); University of California, San Francisco (San Francisco, CA); University of Washington/Virginia Mason Medical Center (Seattle, WA); and Virginia Commonwealth University (Richmond, VA). All participants provided written informed consent prior to data collection. The subjects' demographic characteristics, anthropomorphic measurements, alcohol consumption, medical history, medication use, clinical tests, and liver biopsy results were prospectively collected; the detailed inclusion and exclusion criteria have previously been described (34,35). Liver biopsies were obtained during the study period as clinically indicated. All protocols, consent forms, and manuals of operations were reviewed by a data safety-monitoring board established by the NIDDK for the NASH CRN and approved by the institutional review board for each site.

### NAFLD Diagnosis

Participants had to meet specific criteria regarding the diagnosis of NAFLD in order to be enrolled in this study as previously published (34). Patients with alcohol consumption of >140 g/week if male or >70 g/week if female in the 2 years prior to screening were excluded. Patients with other etiologies of chronic liver disease were also excluded. For the purpose of enrollment into the observational NAFLD Database Study, the diagnosis of NAFLD was based on the histological diagnosis of NAFLD or cryptogenic cirrhosis or on imaging studies (34). For this study, only patients with liver biopsy data available within 6 months of the clinic data were included.

### Description of Liver Histology Assessment

Liver biopsy slides were stained with hematoxylin-eosin, Masson trichrome, and Perls iron stain. The NASH CRN Pathology Committee reviewed and scored the slides without any knowledge of patient's previous diagnosis, clinical information, or laboratory values or the study for which the biopsy was being

evaluated (36,37). The committee used Brunt modified classification to stage fibrosis: 0 = no fibrosis; 1a = mild, zone 3 perisinusoidal fibrosis; 1b = moderate, zone 3 perisinusoidal fibrosis; 1c = portal/periportal only fibrosis; 2 = zone 3 perisinusoidal and periportal fibrosis; 3 = bridging fibrosis; and 4 = cirrhosis (36,38,39). Advanced fibrosis was defined as stages 3–4. Patients were classified as having no NASH, possible/borderline NASH, or definite NASH as previously described (36). For the purposes of this analysis, patients with possible/borderline NASH were grouped with patients with no NASH.

### Outcome Measures

The primary outcome measure was presence of definite NASH as determined by the Pathology Committee's review of the liver biopsy (36). NASH was defined as presence of steatosis predominantly in zone 3, with varying degrees of lobular inflammation, and classic ballooning degeneration with or without presence of Mallory-Denk bodies and/or peri-sinusoidal fibrosis. The secondary outcome measure was presence of advanced fibrosis on liver biopsy defined as presence of stage 3 or 4 fibrosis on the NASH CRN histologic scoring system (36).

### Covariates

All data analyzed in this study were obtained within 6 months of the liver biopsy. The following variables were included: demographic features (age at enrollment [years], sex, race [white or other], ethnicity [Hispanic/Latino]), clinical data (waist circumference, waist-to-hip ratio, BMI [ $\text{kg}/\text{m}^2$ ], hypertension), laboratory measures (serum alanine [ALT] and aspartate [AST] aminotransferase,  $\gamma$ -glutamyl transpeptidase [GGT], albumin, international normalized ratio [INR], bilirubin, triglyceride, HDL, fasting serum glucose and insulin levels, and complete blood count), and presence of type 2 diabetes.

Diabetes status was based upon previous history of diabetes, use of medications to treat diabetes, and/or fasting plasma glucose  $\geq 126$  mg/dL or a 2-h glucose  $\geq 200$  mg/dL during an oral glucose tolerance test or  $\text{HbA}_{1c} \geq 6.5\%$ , consistent with the American Diabetes Association criteria for the diagnosis of diabetes (40).

### Statistical Analyses

Group comparisons used the two-tailed *t* test for continuous dependent variables

and Fisher exact test for categorical variables. Multivariate logistic regression models were used to analyze the diagnostic power of clinical and biochemical characteristics to predict the presence of NASH and of advanced fibrosis. We selected the predictor variables for the clinical prediction models for NASH and for advanced fibrosis using stepwise logistic regression models derived from a large candidate set of clinical variables by application of the Akaike information criterion (AIC) to select each predictor. Predictors with higher information content were selected in the models for the probabilities of NASH. Since AIC is defined as  $-2\log \text{likelihood} + 2p$ , where  $p$  = number of predictors in a model, models with more predictors are “penalized” by the  $2p$  factor in the AIC. This means that a new predictor must improve the information in the model (AIC) more than enough to overcome the “penalty” assigned for adding a new predictor to the model. This approach results in predictors selected for models on the basis of a standard measure of information content and avoids the difficult multiplicity of comparison problems present when predictor selection uses  $P$  values.

We calculated the cross-validated areas under the receiver operating characteristic curves (AUROCs), positive predictive value (PPV), negative predictive value (NPV), the percent correctly classified, sensitivity, and specificity to measure the diagnostic test characteristics of each model. Next, we calculated low-probability cut points for “not NASH” and “not advanced fibrosis” based on the NPV of 90% (or the largest NPV defined) and high probability cut points for “NASH” and “advanced fibrosis” based on the PPV of 90% (or largest PPV defined). The “gray zone” is defined as the probability scores that fall in between the low- and high-probability cut points. Using these cut points, we calculated the number of correctly and incorrectly classified biopsies and the potential for biopsies spared, as well as the number of patient biopsies falling in between the lower and higher cut points (34).

#### Independent Assessment of Model Performance: AUROC Using Jackknifing and AUROC in Validation Cohort

We used a statistical jackknife procedure to obtain an internal but

independently validated (cross-validated) and thus more realistic estimate of an AUROC. If  $n$  = number of patients, the cross-validated AUROC is obtained by fitting a total of  $n$  different models and obtaining  $n$  independently predicted probabilities (scores) of NASH or advanced fibrosis, each with a sample size of  $n - 1$  obtained by deleting one patient at a time until the  $n$  independent scores have been obtained. These independent scores are used to predict each of the  $n$  outcomes and calculate the jackknifed AUROC (41). The jackknife procedure results in independent, and thus more valid, AUROC estimates, since its predictions are of patient results using models fit to data external to the patient being predicted. While a jackknifed AUROC is superior to an ordinary biased AUROC by virtue of predicting outcomes using models derived from these same outcomes, clinical prediction models, to be useful, must be validated in a population that is external to the population used to develop the model. We approximated this using our model for predicting advanced fibrosis in future NASH CRN patients as read by a pathologist serving each clinic rather than the central consensus reading at the histology reading center for the NASH CRN. The model for advanced fibrosis was externally validated using a cohort of NASH CRN patients not included in the primary analysis. This validation cohort consisted of 100 patients from the same studies and time period as used for the model development based upon central review of cases. Finally, we compared our clinical model for advanced fibrosis with a published model for predicting advanced fibrosis by applying this model to our data and comparing the cross-validated AUROCs, PPV, NPV, specificity, and sensitivity.

$P$  values were considered statistically significant if  $P < 0.05$ . All analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC) and Stata 13.1 (StataCorp, College Station, TX).

## RESULTS

### Baseline Data

This study included 1,249 patients with biopsy-proven NAFLD; 435 (34.8%) of these patients had diabetes. The average age and BMI of these patients with diabetes and NAFLD was  $52.4 \pm 10.3$  years and  $35.8 \pm 6.6$  kg/m<sup>2</sup>,

respectively. Among the 346 patients with diabetes and NAFLD, the prevalence of NASH and advanced fibrosis was 69.2% and 41.0% (Table 1). Detailed baseline characteristics of the patients with diabetes are shown in Table 1.

### Predictors of Presence of NASH in Patients With Diabetes

#### Univariate Analysis

Table 1 describes the baseline characteristics of patients with diabetes classified by the presence or absence of definite NASH. In univariate models, factors associated with presence of NASH on histology included elevated AST or ALT ( $P < 0.0001$ ), serum insulin ( $P = 0.001$ ), HbA<sub>1c</sub> ( $P = 0.006$ ), and HOMA of insulin resistance (HOMA-IR) ( $P = 0.002$ ).

#### Multivariable-Adjusted Analyses

A multivariable-adjusted model (clinical model) was developed using AIC criteria. In the clinical model (Table 2), the factors associated with presence of NASH on histology included white race, BMI, waist (measured in centimeters), ALT, AST, albumin, HbA<sub>1c</sub>, HOMA-IR, and ferritin with a cross-validated AUROC of 0.80 (95% CI 0.75–0.84,  $P$  value = 0.007). The specificity, sensitivity, NPVs, and PPVs were 90.0%, 56.8%, 47.7%, and 93.2%, respectively, and this model correctly classified 67% of patients as having NASH (Table 2).

### Predictors of Advanced Fibrosis

#### Univariate Analysis

Table 1 describes the baseline characteristics of patients with diabetes stratified by the presence of advanced fibrosis. In univariate models, the factors associated with advanced fibrosis included age ( $P < 0.0001$ ), hypertension ( $P = 0.006$ ), elevated AST ( $P = 0.02$ ), AST-to-ALT ratio ( $P < 0.0001$ ), GGT ( $P = 0.0006$ ), globulin ( $P = 0.01$ ), direct bilirubin ( $P = 0.01$ ), serum insulin ( $P = 0.002$ ), HOMA-IR ( $P = 0.003$ ), LDL ( $P = 0.05$ ), white blood cell count ( $P = 0.002$ ), INR ( $P = 0.0003$ ), and platelet count ( $P < 0.0001$ ).

#### Multivariable-Adjusted Analysis

A multivariable-adjusted model (clinical model) was developed using AIC criteria. In the clinical model (Table 3), the factors associated with advanced fibrosis included age, Hispanic ethnicity, BMI, waist-to-hip ratio, hypertension, AST-to-ALT ratio, alkaline phosphatase, isolated abnormal alkaline

**Table 1—Adult patients with diabetes and NAFLD: baseline characteristics by the presence of NASH and presence of advanced fibrosis**

Characteristics	Presence of NASH		<i>P</i> * (NASH vs. not NASH)	Presence of advanced fibrosis		
	No (n = 105)	Yes (n = 241)		No: none, mild, or moderate (n = 204)	Yes: bridging or cirrhosis (n = 142)	<i>P</i> * (advanced fibrosis vs. not advanced fibrosis)
<b>Demographics</b>						
Male, n (%)	38 (36.2)	68 (28.2)	0.16	62 (30.4)	44 (31.0)	0.91
Age (years), mean ± SD	51.7 ± 10.0	52.8 ± 10.4	0.37	50.3 ± 11.2	55.7 ± 7.9	<0.0001
White, n (%)	83 (79.0)	200 (83.0)	0.45	163 (79.9)	120 (84.5)	0.32
Hispanic, n (%)	8 (7.6)	23 (9.5)	0.68	21 (10.3)	10 (7.0)	0.34
<b>Clinical, n (%)</b>						
Hypertension	26 (24.8)	98 (40.7)	0.005	61 (29.9)	63 (44.4)	0.006
Metabolic syndrome§	76 (72.4)	206 (85.5)	0.006	165 (80.9)	117 (82.4)	0.78
Acanthosis nigricans§§	14 (13.3)	41 (17.0)	0.43	34 (16.7)	21 (14.8)	0.66
<b>Anthropometric, mean ± SD</b>						
BMI (kg/m <sup>2</sup> )	35.1 ± 6.9	36.2 ± 6.8	0.17	35.5 ± 6.6	36.4 ± 7.1	0.24
Waist (cm)	112.3 ± 14.8	113.4 ± 14.7	0.49	112.4 ± 14.0	114.1 ± 15.7	0.28
Waist-to-hip ratio	0.95 ± 0.08	0.95 ± 0.08	0.72	0.95 ± 0.07	0.95 ± 0.08	0.38
<b>Laboratory measures, mean ± SD</b>						
AST (units/L)	36.4 ± 17.0	65.6 ± 46.8	<0.0001	52.6 ± 45.2	62.8 ± 37.2	0.02
ALT (units/L)	51.5 ± 33.5	80.9 ± 59.8	<0.0001	71.9 ± 59.6	72.0 ± 47.3	0.98
AST-to-ALT ratio	0.82 ± 0.37	0.88 ± 0.32	0.12	0.78 ± 0.28	0.98 ± 0.389	<0.0001
Alkaline phosphatase (units/L)	86.9 ± 35.4	95.2 ± 41.3	0.06	86.8 ± 31.4	101.0 ± 49.2	0.002
Isolated abnormal alkaline phosphatase§§§	9 (8.6)	6 (2.5)	0.02	8 (3.9)	7 (4.9)	0.79
GGT (units/L)	70.4 ± 94.2	98.7 ± 124.0	0.02	70.7 ± 86.7	118.0 ± 144.8	0.0006
Globulin (g/dL)	3.06 ± 0.52	3.13 ± 0.53	0.25	3.05 ± 0.52	3.19 ± 0.54	0.01
Albumin (g/dL)	4.15 ± 0.41	4.21 ± 0.43	0.22	4.26 ± 0.42	4.16 ± 0.43	0.18
Total bilirubin (mg/dL)	0.71 ± 0.43	0.67 ± 0.31	0.42	0.66 ± 0.36	0.71 ± 0.34	0.17
Direct bilirubin (mg/dL)	0.15 ± 0.11	0.15 ± 0.08	0.84	0.14 ± 0.08	0.16 ± 0.10	0.01
INR	1.02 ± 0.20	1.05 ± 0.22	0.28	1.00 ± 0.19	1.09 ± 0.23	0.0003
Hematocrit (%)	40.5 ± 3.9	41.1 ± 3.9	0.74	41.2 ± 3.8	40.4 ± 4.0	0.06
White blood cells (1,000/mm <sup>3</sup> )	7.18 ± 2.3	7.23 ± 2.1	0.87	7.51 ± 2.1	6.79 ± 2.1	0.002
Platelet count (1,000/mm <sup>3</sup> )	240.1 ± 80.7	223.9 ± 71.9	0.08	252.8 ± 71.1	194.3 ± 66.5	<0.0001
Total cholesterol (mg/dL)	178.2 ± 41.1	192.1 ± 44.3	0.005	191.1 ± 44.4	183.3 ± 42.4	0.10
HDL cholesterol (mg/dL)	41.5 ± 11.2	41.7 ± 10.7	0.88	41.2 ± 10.3	42.2 ± 11.6	0.37
LDL cholesterol (mg/dL)	105.8 ± 34.6	114.2 ± 36.5	0.04	114.8 ± 36.5	107.2 ± 35.2	0.05
Triglycerides (mg/dL)	164.9 ± 76.7	190.0 ± 93.3	0.01	187.4 ± 88.0	175.2 ± 90.7	0.21
HbA <sub>1c</sub> (%)	6.8 ± 1.2	7.4 ± 1.3	0.006	7.14 ± 1.31	7.25 ± 1.28	0.93
HbA <sub>1c</sub> (mmol/L)	50.9 ± 13.2	56.9 ± 14.2	0.0003	54.6 ± 14.3	55.7 ± 14.0	0.45
Serum glucose (mg/dL)	124.7 ± 38.2	138.6 ± 52.3	0.006	132.3 ± 48.1	137.4 ± 49.9	0.35
Serum insulin (μU/mL)	23.7 ± 15.9	32.5 ± 34.4	0.001	25.3 ± 23.1	36.4 ± 37.4	0.002
HOMA-IR (mg/dL × μU/mL/405)	7.2 ± 5.0	11.4 ± 13.7	0.002	8.42 ± 9.0	12.6 ± 14.8	0.003
Ferritin (ng/mL)	166.3 ± 169.2	249.1 ± 322.9	0.002	212.6 ± 269.7	240.2 ± 311.5	0.39
<b>Histology, n (%)</b>						
Steatosis ≥34%	48 (45.7)	153 (63.5)	0.003	131 (64.2)	70 (49.3)	0.008
Lobular inflammation ≥grade 2	29 (27.6)	143 (59.3)	<0.0001	100 (49.0)	72 (50.7)	0.50
Ballooning: any	25 (23.8)	241 (100.0)	<0.0001	135 (66.2)	131 (92.2)	<0.0001
Fibrosis stage: bridging or cirrhosis	21 (20.0)	121 (50.2)	<0.0001	102 (50.0)	84 (59.2)	0.03
NAS, mean ± SD	3.06 ± 1.12	5.39 ± 1.32	<0.001	4.52 ± 1.70	4.92 ± 1.56	0.09
NAS ≥5, n (%)	11 (10.5)	175 (72.6)	<0.0001	120 (58.8)	121 (85.2)	<0.0001

Note: patients are from the NASH CRN cohort studies (Database and DB2) enrolled between September 2004 and December 2012. Diagnosis of definite NASH and advanced fibrosis was determined by central review of liver biopsies by the NASH CRN Pathology Committee. NAS, NAFLD activity score. \**P* values determined from Fisher exact test for categorical variables or from *t* test for continuous variables. §National Cholesterol Education Program definition. §§0 = absent, 1 = present on close inspection, 2 = mild, 3 = moderate, 4 = severe. §§§Defined as alkaline phosphatase ≥1 upper limit of normal (ULN), AST < 1 ULN, and ALT < 1 ULN according to local reference ranges.

phosphatase, globulin, albumin, bilirubin (total and direct), serum insulin, hematocrit, INR, and platelet count with a cross-validated AUROC of 0.80 (95% CI 0.76–0.85). The specificity,

sensitivity, NPV, and PPV were 90%, 57%, 75.1%, and 80.2%, respectively, and this model correctly classified 76.6% of patients as having advanced fibrosis (Table 3).

#### Clinical Application of Proposed Models for NASH and for Advanced Fibrosis

Table 4 provides the probability of presence of NASH and advanced fibrosis at



**Table 2—Clinical model for NASH in adult patients with diabetes and NAFLD**

Characteristics (n = 346)	Clinical model*		
	OR	95% CI	P
<b>Demographics</b>			
White versus nonwhite	1.76	0.86–3.60	0.12
<b>Obesity measures</b>			
BMI (kg/m <sup>2</sup> )	1.11	1.03–1.20	0.006
Waist (cm)	0.97	0.93–0.999	0.04
<b>Laboratory measures</b>			
AST (units/L)	1.07	1.04–1.10	<0.001
ALT (units/L)	0.98	0.97–0.998	0.03
Albumin (g/dL)	2.03	0.96–4.30	0.06
HbA <sub>1c</sub> (%)	1.27	0.93–1.64	0.06
HOMA-IR (mg/dL × μU/mL/405)	1.06	1.01–1.09	0.18
Ferritin (ng/mL)	1.001	1.000–1.003	0.04
<b>Model performance</b>			
Cross-validated AUROC	0.80	0.75–0.84	
PPV	93.2%		
NPV	47.7%		
Correctly classified	67.0%		
Sensitivity	56.8%		
Specificity (fixed at 90%)	90.0%		
AIC	342.2		
Population prevalence of NASH	70%		
Probability cutoff for NASH†	≥0.77		

Clinical model for P (probability of NASH). Coefficients and SEs shown as b(SE):  $\log(P/1 - P) = -7.00(2.47) + 0.106(0.039) \times \text{BMI (kg/m}^2) - 0.035(0.017) \times \text{waist (cm)} + 0.068(0.012) \times \text{AST (units/L)} - 0.016(0.007) \times \text{ALT (units/L)} + 0.71(0.38) \times \text{albumin (g/dL)} + 0.24(0.13) \times \text{HbA}_{1c} (\%) + 0.057(0.024) \times \text{HOMA-IR (mg/dL} \times \mu\text{U/mL/405)} + 0.0014(0.0007) \times \text{ferritin (ng/dL)} + 0.57(0.36) \text{ if white}$ . PPV: probability that the disease is present when the test is positive; NPV: probability that the disease is not present when the test is negative. \*Logistic regression model variables selected from candidate set of baseline variables using AIC with backward selection to select the model with the highest information from a large candidate set of baseline variables to identify the predictors of NASH in adult patients with diabetes with NAFLD: age, sex, white race, Hispanic ethnicity, hypertension, metabolic syndrome, abnormal alkaline phosphatase, BMI, waist (cm), waist-to-hip ratio, AST, ALT, AST-to-ALT ratio, alkaline phosphatase, albumin, direct bilirubin, total bilirubin, white blood cell count, platelets, GGT, total cholesterol, HDL, LDL, triglycerides, ferritin, INR, serum glucose, serum insulin, globulin, hematocrit, HbA<sub>1c</sub>, and HOMA-IR. †Classify as NASH if the model probability of NASH is  $\geq 0.77$ . This cutoff was chosen to give a specificity of 0.90.

various cut points. It also shows the cut points that could be used in clinical practice to determine when to consider a biopsy for the diagnosis of NASH; a model parameter of  $>0.75$  would result in a PPV of 90% for the presence of NASH. Similarly, for advanced fibrosis, a cut point  $>0.85$  would result in a PPV of 89.5% for advanced fibrosis.

#### Internal Cross-Validation and External Validation

Internal cross-validation was done and is shown in Table 3 using jackknife procedures (as explained in RESEARCH DESIGN AND METHODS). Using an independent validation cohort of 100 patients recruited from the NASH CRN sites as part of the same studies, we showed that the results remained consistently robust with AUROC for NASH and advanced fibrosis in the validation cohort of 0.83 (95% CI

0.75–0.92) and 0.84 (0.76–0.92), respectively (as shown in Table 5).

#### Comparison Between the Proposed Diabetes-Specific Model and NAFLD Fibrosis Score

Finally, we compared the diagnostic accuracy of the current model (developed specifically for patients with diabetes) with the NAFLD Fibrosis Score (as shown in Supplementary Table 1). The models developed for the diabetic population were significantly more accurate than the previously published NAFLD Fibrosis Score applied to this population for the diagnosis of advanced fibrosis, with a cross-validated AUROC of 0.80 vs. 0.76 ( $P < 0.05$ ).

#### CONCLUSIONS

##### Main Findings

With a large, well-characterized cohort of patients with biopsy-proven

NAFLD and diabetes, we demonstrate that routinely available clinical and biochemical factors can be used to accurately determine the likelihood of NASH (AUROC 0.80,  $P = 0.007$ ) and advanced fibrosis (AUROC 0.80,  $P < 0.001$ ) in patients with diabetes and NAFLD. These data can guide clinicians regarding when to refer patients with diabetes who have NAFLD for a liver biopsy. The application of these prediction models accurately classified 67% of our study set with NASH and 77% with advanced fibrosis. The models are clinically stringent and weighted to having high PPVs with the trade-off of lower NPVs. Thus, clinical judgment and further testing, including liver biopsies, may still be needed in patients determined not to be at high risk for NASH or advanced fibrosis using these models but would correctly classify three-quarters of patients with advanced fibrosis.

Prior studies have used similar clinical and laboratory measures to identify patients with NAFLD to predict the presence or absence of advanced fibrosis in NAFLD patients. One example is the Fatty Liver Index, which uses triglyceride level and waist circumference to predict NAFLD (42,43). Other studies of NAFLD patients have demonstrated that the presence of metabolic syndrome and hypertriglyceridemia, higher AST-to-ALT ratio, and lower platelet count are associated with more advanced liver disease (34,44). Clinical prediction rules have also been created to identify NAFLD patients with and without advanced fibrosis. One example is the well-validated NAFLD fibrosis score, which consists of age, BMI, impaired fasting glucose or diabetes, AST-to-ALT ratio, platelet count, and albumin (45,46). Our model performed better than the NAFLD fibrosis score. Unlike prior studies, the current model proposed in this study focuses on patients with diabetes, a population known to have higher risk of NASH, advanced fibrosis, and mortality (10,26,47–50). The models developed in this study can thus help to identify patients with diabetes at high risk for the presence of NASH or advanced fibrosis and help guide clinicians when to refer patients with diabetes for a liver biopsy and appropriate management. Future studies combining

**Table 3—Clinical model for advanced fibrosis in adult patients with diabetes and NAFLD**

Characteristics (n = 346)	Clinical model*		
	OR	95% CI	P
<b>Demographics</b>			
Age (years)	1.04	1.01–1.07	0.007
Hispanic versus non-Hispanic	0.46	0.16–1.27	0.13
<b>Clinical status</b>			
Hypertension	1.56	0.89–2.73	0.12
<b>Obesity measures</b>			
BMI (kg/m <sup>2</sup> )	1.04	0.998–1.090	0.06
Waist-to-hip ratio	21.2	0.55–821	0.10
<b>Laboratory measures</b>			
AST-to-ALT ratio	3.54	1.27–9.88	0.02
Alkaline phosphatase (units/L)	1.014	1.005–1.024	0.003
Isolated abnormal alkaline phosphatase	0.26	0.05–1.35	0.11
Globulin (g/dL)	2.27	1.26–4.07	0.006
Albumin (g/dL)	3.42	1.44–8.10	0.005
Total bilirubin (mg/dL)	0.44	0.16–1.24	0.12
Direct bilirubin (mg/dL)	24.4	0.47–1.254	0.11
INR	4.74	0.96–23.5	0.06
<b>Hematology and other laboratory studies</b>			
Hematocrit (%)	0.902	0.83–0.98	0.01
Platelet count (1,000/mm <sup>3</sup> )	0.987	0.982–0.991	<0.001
Serum insulin (μU/mL)	1.013	1.002–1.024	0.02
<b>Model performance</b>			
Cross-validated AUROC	0.803	0.756–0.850	
PPV	80.2%		
NPV	75.1%		
Correctly classified	76.6%		
Sensitivity	57.0%		
Specificity (fixed at 90%)	90.0%		
AIC	368.4		
Probability cutoff for advanced fibrosis†	≥0.60		

Clinical model for P (probability of advanced fibrosis). Coefficients and SEs shown as b(se):  $\log(P/1 - P) = -11.8(3.8) + 0.04(0.015) \times \text{age (years)} + 0.042(0.023) \times \text{BMI (kg/m}^2\text{)} + 3.05(1.87) \times \text{waist-to-hip ratio} + 0.014(0.005) \times \text{ALK (units/L)} + 1.26(0.52) \times \text{AST-to-ALT ratio} + 1.23(0.44) \times \text{albumin (g/dL)} + 0.82(0.30) \times \text{globulin (g/dL)} - 0.103(0.041) \times \text{hematocrit (\%)} - 0.0133(0.0024) \times \text{platelet count (1,000/mm}^3\text{)} + 3.19(2.01) \times \text{direct bilirubin (mg/dL)} - 0.81(0.52) \times \text{total bilirubin (mg/dL)} - 1.33(0.83) \text{ if abnormal alkaline phosphatase} + 1.56(0.82) \times \text{INR} + 0.0131(0.0056) \times \text{serum insulin (}\mu\text{U/mL)} - 0.79(0.52) \text{ if Hispanic} + 0.44(0.28) \text{ if hypertensive. ALK: alkaline phosphatase; PPV: probability that the disease is present when the test is positive; NPV: probability that the disease is not present when the test is negative. *Logistic regression model variables selected from candidate set of baseline variables using AIC with backward selection to select the model with the highest information from a large candidate set of baseline variables to identify the predictors of advanced fibrosis in adult patients with diabetes with NAFLD: age, sex, white race, Hispanic, hypertension, metabolic syndrome, abnormal alkaline phosphatase, BMI, waist (cm), waist-to-hip ratio, AST, ALT, AST-to-ALT ratio, alkaline phosphatase, albumin, direct bilirubin, total bilirubin, white blood cell count, platelets, GGT, total cholesterol, HDL, LDL, triglycerides, ferritin, INR, serum glucose, serum insulin, globulin, hematocrit, HbA<sub>1c</sub>, and HOMA-IR. †Classify as advanced fibrosis if the model probability of advanced fibrosis is  $\geq 0.60$ . This cutoff was chosen to give a specificity of 0.90.$

the clinical prediction rules with other noninvasive imaging methods (51) need to be performed to further improve the diagnostic accuracy.

### Strengths and Limitations

The NASH CRN cohort is a multiethnic and multicenter study including eight sites across the U.S. This ethnic and geographic variation is a strength that may allow the results to be applied to other NAFLD patients in the U.S. Additionally,

the NASH CRN cohort includes prospective cohort data. The histology was also subject to blinded analysis by a committee of expert pathologists who used the accepted and validated NASH CRN histology scoring system. A series of validation procedures were used to confirm the reproducibility of findings. We performed jackknife internal cross-validation of AUROC, as it is superior to an ordinary biased AUROC. In order for the models to

be generalizable, they must be validated in a population that is external to the population used to develop the model. We approximated this using our model for predicting advanced fibrosis in future NASH CRN patients as read by a pathologist serving each clinic rather than the central consensus reading at the histology reading center for the NASH CRN. The model for advanced fibrosis was externally validated using a cohort of NASH CRN patients not included in the primary analysis. This validation cohort consisted of 100 patients from the same studies and time period as used for the model development based upon central review of cases. The results remained statistically significant and robust and consistent with the model development cohort. One limitation of the study is the recruitment of patients from tertiary care centers. The associations between diabetes-related phenotypes—diabetes or diabetes-related treatments, such as, but not exclusively, insulin, metformin, sulfonylureas, or statins and fibrates and the duration of diabetes—and their possible effects on the development of NASH and advanced fibrosis were not examined. This study population may not represent the spectrum of patients in the general population seen in primary care. Further studies would be needed to externally validate these results.

### Implications for Future Research and Clinical Practice

This study may help to guide further research on potential relationships between NASH and diabetes. It may help identify high-risk patients and target interventions in order to prevent progression of NASH to cirrhosis and hepatocellular carcinoma, especially in populations with diabetes.

Currently, NAFLD Practice Guidelines recommend the use of the NAFLD Fibrosis Score for the screening for advanced fibrosis. In this report, we demonstrate that the proposed model is better than NAFLD Fibrosis Score in assessing advanced fibrosis in patients with diabetes. If validated in an independent cohort, the current model will replace the NAFLD fibrosis score in the NAFLD Practice Guidelines in future. These data could also be used to screen patients with diabetes who should be screened for NASH prior to

**Table 4—Application of clinical models for NASH and advanced fibrosis in patients with diabetes and NAFLD**

NASH clinical model	Probabilities of NASH			Total
	Not NASH ( $P < 0.33$ )	Gray zone ( $0.33 \leq P \leq 0.75$ )	NASH ( $P > 0.75$ )	
Total patients	30	153	163	346
NASH present				
Yes	7	85	149	241
No	23	68	14	105
Potential for biopsies spared by application of the model	8.7% (30/346)		47.1% (163/346)	55.8% (193/346)
Advanced fibrosis clinical model	Probabilities of advanced fibrosis			Total
	Not advanced fibrosis ( $P < 0.023$ )	Gray zone ( $0.023 \leq P \leq 0.85$ )	Advanced fibrosis ( $P > 0.85$ )	
Total patients	10	300	36	346
Advanced fibrosis present				
Yes	0	108	34	142
No	10	5,192	2	204
Potential for biopsies spared by application of the model	2.9% (10/346)		10.4% (36/346)	13.3% (46/346)

Data are  $n$  unless otherwise indicated. The model probability cutoff of 0.75 for NASH and the probability cutoff of 0.85 for advanced fibrosis were selected to attain a PPV of 90%. Application rule for NASH: do not biopsy if the probability of NASH is  $>0.75$  (assume NASH) or  $<0.33$  (assume not NASH). Application rule for advanced fibrosis: do not biopsy if the probability of advanced fibrosis is  $>0.85$  (assume advanced fibrosis) or  $<0.023$  (assume not advanced fibrosis). Note: the performance of these models varies with the prevalence of NASH (70%) and the prevalence of advanced fibrosis (41%) in the population.

enrollment in a clinical trial. This is emerging to be an important unmet need, and these findings provide a clinically useful

tool that can be applied directly in clinical practice using routinely available data.

## Conclusion

Using a large, diverse cohort of patients with biopsy-proven NAFLD and diabetes, we developed a clinical prediction guide to identify patients with diabetes at risk for having NASH using readily available clinical data such as BMI, presence of hypertension, and routine laboratory values. This guide could potentially impact an estimated 10 million people residing in the U.S. who have coexisting diabetes and NASH by allowing for early identification of high-risk patients. These models may help inform the decision as to who should be considered for liver biopsy and/or referred to a hepatologist for further evaluation of NAFLD. Further studies using additional biomarkers are needed to improve the clinical models and to better understand the pathogenesis of NASH and its relationship with diabetes.

**Funding.** This work was supported in part by the American Gastroenterological Association (AGA) Foundation–Sucampo–Association of Specialty Professors (ASP) Designated Research Award in Geriatric Gastroenterology and by a T. Franklin Williams Scholarship Award. Funding was also provided by The Atlantic Philanthropies, the John A. Hartford Foundation, the ASP, the AGA (to R.L.), and the NIDDK, National Institutes of Health (grant K23-DK-090303). This research was funded in part with the support of the University of California, San Diego, Digestive

**Table 5—Validation of clinical models for NASH and for advanced fibrosis in external population**

	NASH		<i>P</i>
	Model development cohort	Model validation cohort*	
Number of patients	346	100	
AUROC (95% CI)	0.82 (0.77–0.87)	0.83 (0.75–0.92)	0.76
PPV	93.4%	90.9%	0.39
NPV	43.8%	47.8%	0.48
Sensitivity	90.3%	91.4%	0.74
Specificity	54.7%	46.2%	0.13
Correctly classified	64.7%	62.0%	0.62
Prevalence	70%	65%	0.34
	Advanced fibrosis		<i>P</i>
	Model development cohort	Model validation cohort*	
Number of patients	346	100	
AUROC (95% CI)	0.84 (0.80–0.88)	0.84 (0.76–0.92)	0.97
PPV	80.2%	74.2%	0.20
NPV	75.1%	78.3%	0.51
Sensitivity	57.0%	60.5%	0.53
Specificity	90.0%	87.1%	0.41
Correctly classified	76.6%	77.0%	0.93
Prevalence	41%	38%	0.59

\*The validation data set consists of data from future NASH CRN patients as read by a pathologist serving each clinic rather than the central, consensus reading at the histology reading center for the NASH CRN. These patients were not included in the primary analysis. This validation cohort consists of 100 patients from the same studies and time period as used for the model development based upon central review of cases.



Diseases Research Development Center, Public Health Service grant DK-080506. NASH CRN is supported by the NIDDK (grants U01-DK-061718, U01-DK-061728, U01-DK-061731, U01-DK-061732, U01-DK-061734, U01-DK-061737, U01-DK-061738, U01-DK-061730, and U01-DK-061713), by the Eunice Kennedy Shriver National Institute of Child Health and Human Development, and by the National Institutes of Health.

**Duality of Interest.** No potential conflicts of interest relevant to this article were reported.

**Author Contributions.** J.B. and R.L. drafted the manuscript. M.D., L.W., J.T., and R.L. analyzed and interpreted data. M.D., B.A.N.-T., D.K., E.M.B., L.W., E.D., J.L., and J.T. critically revised the manuscript for important intellectual content. M.D., L.W., J.T., and R.L. performed statistical analysis. R.L. conceived the study concept and design, acquired data, provided technical or material support, and supervised the study. R.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

## References

- Younossi ZM, Stepanova M, Afendy M, Fang Y, Younossi Y, Mir H, Srishord M. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. *Clin Gastroenterol Hepatol* 2011;9:524–530
- Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am J Gastroenterol* 2003;98:960–967
- Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003;37:1202–1219
- Rafiq N, Bai C, Fang Y, et al. Long-term follow-up of patients with nonalcoholic fatty liver. *Clin Gastroenterol Hepatol* 2009;7:234–238
- Söderberg C, Stål P, Askling J, et al. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. *Hepatology* 2010;51:595–602
- Adams LA, Lymp JF, St Sauver J, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005;129:113–121
- Ascha MS, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology* 2010;51:1972–1978
- Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* 2013;10:686–690
- Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver versus nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol* 2015;13:643–654
- Stepanova M, Aquino R, Alsheddi A, Gupta R, Fang Y, Younossi Z. Clinical predictors of fibrosis in patients with chronic liver disease. *Aliment Pharmacol Ther* 2010;31:1085–1094
- Suzuki A, Angulo P, Lymp J, et al. Chronological development of elevated aminotransferases in a nonalcoholic population. *Hepatology* 2005;41:64–71
- Doycheva I, Patel N, Peterson M, Loomba R. Prognostic implication of liver histology in patients with nonalcoholic fatty liver disease in diabetes. *J Diabetes Complications* 2013;27:293–300
- Mokdad AH, Bowman BA, Ford ES, Vinicor F, Marks JS, Koplan JP. The continuing epidemics of obesity and diabetes in the United States. *JAMA* 2001;286:1195–1200
- Knowler WC, Barrett-Connor E, Fowler SE, et al.; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002;346:393–403
- Centers for Disease Control and Prevention. *National Diabetes Fact Sheet: National Estimates and General Information on Diabetes and Prediabetes in the United States, 2011*. Atlanta, GA, Centers for Disease Control and Prevention, 2011
- Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011;34:274–285
- Williams CD, Stengel J, Asike MI, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011;140:124–131
- Wong VW, Chan HL, Hui AY, et al. Clinical and histological features of non-alcoholic fatty liver disease in Hong Kong Chinese. *Aliment Pharmacol Ther* 2004;20:45–49
- Amarapurkar D, Kamani P, Patel N, et al. Prevalence of non-alcoholic fatty liver disease: population based study. *Ann Hepatol* 2007;6:161–163
- Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology* 2005;42:44–52
- Williamson RM, Price JF, Glancy S, et al.; Edinburgh Type 2 Diabetes Study Investigators. Prevalence of and risk factors for hepatic steatosis and nonalcoholic fatty liver disease in people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabetes Care* 2011;34:1139–1144
- Targher G, Bertolini L, Padovani R, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 2007;30:1212–1218
- Lazo M, Hernaez R, Eberhardt MS, et al. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988–1994. *Am J Epidemiol* 2013;178:38–45
- Ortiz-Lopez C, Lomonaco R, Orsak B, et al. Prevalence of prediabetes and diabetes and metabolic profile of patients with nonalcoholic fatty liver disease (NAFLD). *Diabetes Care* 2012;35:873–878
- Prashanth M, Ganesh HK, Vima MV, et al. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. *J Assoc Physicians India* 2009;57:205–210
- Hamaguchi M, Kojima T, Takeda N, et al. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. *Ann Intern Med* 2005;143:722–728
- Ong JP, Elariny H, Collantes R, et al. Predictors of nonalcoholic steatohepatitis and advanced fibrosis in morbidly obese patients. *Obes Surg* 2005;15:310–315
- Chalasani N, Younossi Z, Lavine JE, et al.; American Association for the Study of Liver Diseases; American College of Gastroenterology; American Gastroenterological Association. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Am J Gastroenterol* 2012;107:811–826
- Boursier J, Rousselet MC, Aubé C, Calès P. Liver fibrosis in patients with non-alcoholic fatty liver disease: diagnostic options in clinical practice. *Expert Opin Med Diagn* 2012;6:381–394
- Grandison GA, Angulo P. Can NASH be diagnosed, graded, and staged noninvasively? *Clin Liver Dis* 2012;16:567–585
- Paredes AH, Torres DM, Harrison SA. Non-alcoholic fatty liver disease. *Clin Liver Dis* 2012;16:397–419
- Stinton LM, Loomba R. Recommendations for liver biopsy evaluation in non-alcoholic fatty liver disease. *Minerva Gastroenterol Dietol* 2014;60:5–13
- Chalasani NP, Sanyal AJ, Kowdley KV, et al.; NASH CRN Research Group. Pioglitazone versus vitamin E versus placebo for the treatment of non-diabetic patients with non-alcoholic steatohepatitis: PIVENS trial design. *Contemp Clin Trials* 2009;30:88–96
- Neuschwander-Tetri BA, Clark JM, Bass NM, et al.; NASH Clinical Research Network. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. *Hepatology* 2010;52:913–924
- Sanyal AJ, Chalasani N, Kowdley KV, et al.; NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010;362:1675–1685
- Kleiner DE, Brunt EM, Van Natta M, et al.; Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;41:1313–1321
- Brunt EM, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA; NASH Clinical Research Network (CRN). Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. *Hepatology* 2011;53:810–820
- Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Non-alcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999;94:2467–2474
- Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999;116:1413–1419
- Olson DE, Rhee MK, Herrick K, Ziemer DC, Twombly JG, Phillips LS. Screening for diabetes and pre-diabetes with proposed A1C-based diagnostic criteria. *Diabetes Care* 2010;33:2184–2189

41. Rabe-Hesketh S, Everitt BS. *A Handbook of Statistical Analyses Using Stata*. 4th ed. Florida, Chapman and Hall, 2007, p. 128
42. Bedogni G, Bellentani S, Miglioli L, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006;6:33
43. Zelber-Sagi S, Webb M, Assy N, et al. Comparison of fatty liver index with noninvasive methods for steatosis detection and quantification. *World J Gastroenterol* 2013;19:57–64
44. Loomba R, Abraham M, Unalp A, et al.; Non-alcoholic Steatohepatitis Clinical Research Network. Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. *Hepatology* 2012;56:943–951
45. Castera L, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. *Nat Rev Gastroenterol Hepatol* 2013;10:666–675
46. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007;45:846–854
47. Adams LA, Harmsen S, St Sauver JL, et al. Nonalcoholic fatty liver disease increases risk of death among patients with diabetes: a community-based cohort study. *Am J Gastroenterol* 2010;105:1567–1573
48. Harrison SA, Torgerson S, Hayashi PH., The natural history of nonalcoholic fatty liver disease: a clinical histopathological study. *Am J Gastroenterol* 1999;98:2042–2047
49. de Marco R, Locatelli F, Zoppini G, Verlato G, Bonora E, Muggeo M. Cause-specific mortality in type 2 diabetes. The Verona Diabetes Study. *Diabetes Care* 1999;22:756–761
50. Zarrinpar A, Loomba R. Review article: the emerging interplay among the gastrointestinal tract, bile acids and incretins in the pathogenesis of diabetes and non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2012;36:909–921
51. Loomba R, Wolfson T, Ang B, et al. Magnetic resonance elastography predicts advanced fibrosis in patients with nonalcoholic fatty liver disease: a prospective study. *Hepatology* 2014;60:1920–1928